

## CLAIMS

We claim:

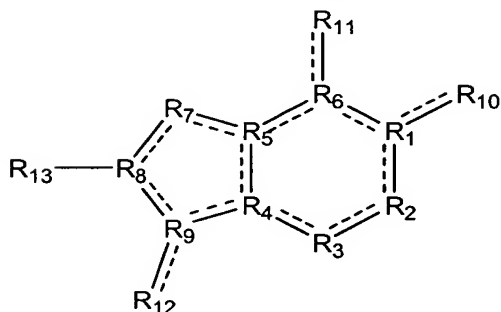
1. A regulatable gene expression construct comprising  
a nucleic acid molecule encoding an RNA comprising a riboswitch operably linked to a coding region, wherein the riboswitch regulates expression of the RNA, wherein the riboswitch and coding region are heterologous.
2. The construct of claim 1 wherein the riboswitch comprises an aptamer domain and an expression platform domain, wherein the aptamer domain and the expression platform domain are heterologous.
3. The construct of claim 1 wherein the riboswitch comprises an aptamer domain and an expression platform domain, wherein the aptamer domain comprises a P1 stem, wherein the P1 stem comprises an aptamer strand and a control strand, wherein the expression platform domain comprises a regulated strand, wherein the regulated strand, the control strand, or both have been designed to form a stem structure.
4. A riboswitch, wherein the riboswitch is a non-natural derivative of a naturally-occurring riboswitch.
5. The riboswitch of claim 4 wherein the riboswitch comprises an aptamer domain and an expression platform domain, wherein the aptamer domain and the expression platform domain are heterologous.
6. The riboswitch of claim 4 wherein the riboswitch is derived from a naturally-occurring guanine-responsive riboswitch, adenine-responsive riboswitch, lysine-responsive riboswitch, thiamine pyrophosphate-responsive riboswitch, adenosylcobalamin-responsive riboswitch, flavin mononucleotide-responsive riboswitch, or a S-adenosylmethionine-responsive riboswitch.
7. The riboswitch of claim 4 wherein the riboswitch is activated by a trigger molecule, wherein the riboswitch produces a signal when activated by the trigger molecule.
8. A method of detecting a compound of interest, the method comprising  
bringing into contact a sample and a riboswitch, wherein the riboswitch is activated by the compound of interest, wherein the riboswitch produces a signal when activated by the compound of interest, wherein the riboswitch produces a signal when the sample contains the compound of interest.

9. The method of claim 8 wherein the riboswitch changes conformation when activated by the compound of interest, wherein the change in conformation produces a signal via a conformation dependent label.

10. The method of claim 8 wherein the riboswitch changes conformation when activated by the compound of interest, wherein the change in conformation causes a change in expression of an RNA linked to the riboswitch, wherein the change in expression produces a signal.

11. The method of claim 10 wherein the signal is produced by a reporter protein expressed from the RNA linked to the riboswitch.

12. A method of inhibiting gene expression, the method comprising bringing into contact a compound and a cell, wherein the compound has the structure



wherein, when the compound is bound to a guanine-responsive riboswitch, R<sub>7</sub> serves as a hydrogen bond acceptor, R<sub>10</sub> serves as a hydrogen bond donor, R<sub>11</sub> serves as a hydrogen bond acceptor, R<sub>12</sub> serves as a hydrogen bond donor,

wherein R<sub>13</sub> is H, H<sub>2</sub> or is not present,

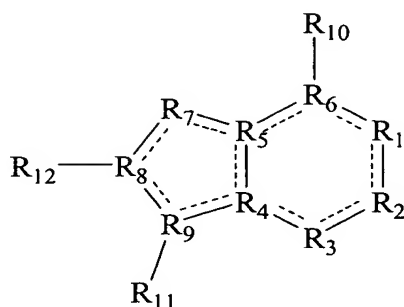
wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>8</sub>, and R<sub>9</sub> are each independently C, N, O, or S,

wherein ----- each independently represent a single or double bond,

wherein the compound is not guanine, hypoxanthine, or xanthine,

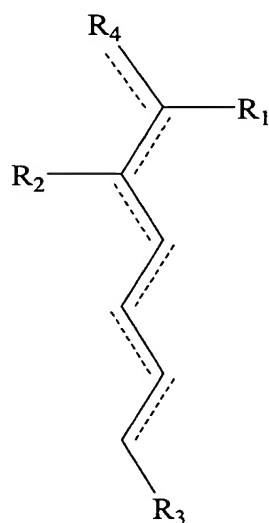
wherein the cell comprises a gene encoding an RNA comprising a guanine-responsive riboswitch, wherein the compound inhibits expression of the gene by binding to the guanine-responsive riboswitch.

13. A method of inhibiting gene expression, the method comprising bringing into contact a compound and a cell, wherein the compound has the structure



wherein, when the compound is bound to an adenine-responsive riboswitch, R<sub>1</sub>, R<sub>3</sub> and R<sub>7</sub> serve as hydrogen bond acceptors, and R<sub>10</sub> and R<sub>11</sub> serve as hydrogen bond donors, wherein R<sub>12</sub> is H, H<sub>2</sub> or is not present, wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>8</sub>, and R<sub>9</sub> are each independently C, N, O, or S, wherein ----- each independently represent a single or double bond, wherein the compound is not adenine, 2,6-diaminopurine, or 2-amino purine, wherein the cell comprises a gene encoding an RNA comprising an adenine-responsive riboswitch, wherein the compound inhibits expression of the gene by binding to the adenine-responsive riboswitch.

14. A method of inhibiting gene expression, the method comprising bringing into contact a compound and a cell, wherein the compound has the structure



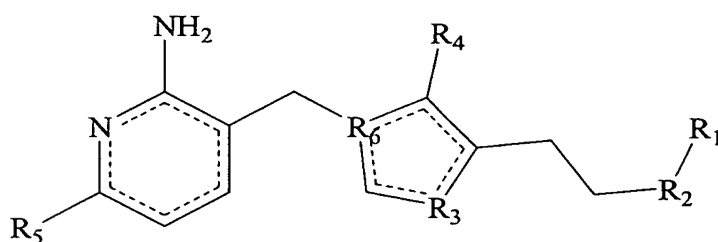
wherein R<sub>2</sub> and R<sub>3</sub> are each positively charged, wherein R<sub>1</sub> is negatively charged, wherein R<sub>4</sub> is C, N, O, or S, wherein ----- each independently represent a single or double bond,

wherein the compound is not lysine,

wherein the cell comprises a gene encoding an RNA comprising a lysine-responsive riboswitch, wherein the compound inhibits expression of the gene by binding to the lysine-responsive riboswitch.

15. The method of claim 14 wherein  $R_2$  and  $R_3$  are each  $\text{NH}_3^+$  and wherein  $R_1$  is  $\text{O}^-$ .

16. A method of inhibiting gene expression, the method comprising bringing into contact a compound and a cell, wherein the compound has the structure



wherein  $R_1$  is positively charged,

wherein  $R_2$  and  $R_3$  are each independently C, O, or S,

wherein  $R_4$  is  $\text{CH}_3$ ,  $\text{NH}_2$ , OH, SH, H or not present,

wherein  $R_5$  is  $\text{CH}_3$ ,  $\text{NH}_2$ , OH, SH, or H,

wherein  $R_6$  is C or N,

wherein ----- each independently represent a single or double bond,

wherein the compound is not TPP, TP or thiamine,

wherein the cell comprises a gene encoding an RNA comprising a thiamine pyrophosphate -responsive riboswitch, wherein the compound inhibits expression of the gene by binding to the thiamine pyrophosphate-responsive riboswitch.

17. The method of claim 16 wherein  $R_1$  is phosphate, diphosphate or triphosphate.

18. A method comprising

(a) testing a compound for inhibition of gene expression of a gene encoding an RNA comprising a riboswitch, wherein the inhibition is via the riboswitch,

(b) inhibiting gene expression by bringing into contact a cell and a compound that inhibited gene expression in step (a),

wherein the cell comprises a gene encoding an RNA comprising a riboswitch, wherein the compound inhibits expression of the gene by binding to the riboswitch.

19. A method of identifying riboswitches, the method comprising

assess in-line spontaneous cleavage of an RNA molecule in the presence and absence of a compound, wherein the RNA molecule is encoded by a gene regulated by the compound, wherein a change in the pattern of in-line spontaneous cleavage of the RNA molecule indicates a riboswitch.